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DEPARTMENT OF THE ARMY
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D.C. 20314

REPLY TO ATTENTION OF:
SGRD-RM

5 December 1977

SUBJECT: Request for Quotation (RFQ) DAMD 17-78-Q-8390 "Studies of Environmental Fates of Diisopropyl Methylphosphonate and Decyclopentadiene"

TO: SOURCE SELECTION BOARD MEMBERS

1. Inclosed are copies of the 7 quotes received in response to subject RFQ, along with a copy of the RFQ. In order to avoid any influence that proposed cost information might have on the technical merits of proposals evaluated, this information has been withdrawn. As a Board member, you are responsible for rating the proposals in accordance with the criteria set out in the RFQ and evaluation sheets. Minor clarifications required on any information contained in the proposals should be referred to the Contracting Officer's Representative who may contact quoters. Personnel participating in any way in evaluating proposals shall not reveal any information (number and names of quoters, scoring or ranking, contents of proposals) concerning the procurement to any individual who is not also participating in the same evaluation procedings. Divulging information during the evaluation, selection, and negotiation phases of the procurement to personnel not having a need to know could jeopardize any resultant award.

2. Each proposal will be independently reviewed and classified as acceptable or unacceptable. A proposal should be considered acceptable if it (1) addresses itself to all essential elements of the RFQ, (2) shows the quoter understands all essential requirements of the RFQ, and (3) the proposal is complete to the extent that a virtually new proposal is not required. A proposal should be considered unacceptable if it is not capable of being made acceptable by meaningful negotiations, or if it shows the contractor does not understand the essential requirements of the RFQ. There must be concrete technical reasons to support unacceptability.

3. At the meeting of the Board, discussions will take place on the strengths and weaknesses of the proposals, and previous experience with the quoters. Cost data will then be provided Board members, and the competitive range established. The competitive range should include all quotes that merely satisfy minimum standards to qualify. Should there be doubt as to whether or not a proposal is in the competitive range, it will be included. The technically superior proposal(s), with the most realistic cost estimate(s), will be determined by vote or consensus of the Board, and recommended to the contracting officer for final negotiations.

SGRD-RM

SUBJECT: Request for Quotation (RFQ) DAMD 17 78-Q-8390 "Studies of Environmental Fates of Diisopropyl Methylphosphonate and Decyclopentadiene"

4. The President of the Board will notify you of the date and place for the Source Selection Meeting. We look forward to seeing you then, and if in the meantime you have any questions or we may assist you in any way, please do not hesitate to call the undersigned at (202) 693-8360.

Sincerely,

Ann E. May

Ms. ANN E. MAY
Contracting Officer's
Representative

Incls
as

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	10/24/77	

4. TITLE AND SUBTITLE
STUDIES OF ENVIRONMENTAL FATES OF DIISOPROPYL METHYLPHOSPHONATE AND DICYCLOPENTADIENE
(TECHNICAL PROPOSAL)

5. FUNDING NUMBERS
DAMD17 78 Q 8390

6. AUTHOR(S)

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
SRI INTERNATIONAL
MENLO PARK, CA

8. PERFORMING ORGANIZATION REPORT NUMBER
81340R10

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, DC

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

SIGNIFICANT GROUNDWATER CONTAMINATION HAS BEEN OBSERVED BEYOND THE BOUNDARIES OF RMA. DIISOPROPYL METHYLPHOSPHONATE (DIMP) AND DICYCLOPENTADIENE (DCPD) ARE TWO COMPOUNDS THAT HAVE LEACHED FROM SOILS AND HAVE REACHED GROUNDWATER SUPPLIES.

TO UNDERSTAND THE ENVIRONMENTAL IMPACT OF SUCH CHEMICALS, THEIR PERSISTENCE IN THE ENVIRONMENT MUST BE DETERMINED AND THEIRTRANSFORMATION PRODUCTS THAT RESULT FROM KNOWN ROUTES OF CHEMICAL TRANSFORMATION IN THE ENVIRONMENT ARE PHOTOCHEMICAL OXIDATION AND MICROBIAL BIODEGRADATION. LITTLE INFORMATION EXISTS ABOUT THESE PROCESSES RELATIVE TO DIMP AND DCPD.

THE PROPOSED RESEARCH PROGRAM IS AIMED AT UNDERSTANDING THESE PROCESSES USING LABORATORY CONDITIONS TO PREDICT THE PERSISTENCE OF DIMP AND DCPD AND TO IDENTIFY THEIR TRANSFORMATION PRODUCTS RESULTING FROM PHOTOCHEMICAL AND MICROBIAL PROCESSES. SINCE THE USAMRDC HAS THE RESPONSIBILITY OF MAINTAINING THE ENVIRONMENTAL QUALITY AT MILITARY INSTALLATIONS, THIS INFORMATION WILL AID

14. SUBJECT TERMS
ANALYTICAL CHEMISTRY, PHOTOCHEMISTRY, MICROBIAL STUDIES, RMA, BIODEGRADATION

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED			

SCORE SHEET FOR DAMD17-77-Q-8390
(500 POINT TOTAL)

1. Proposed Methodology - 200
   a. Chemical Studies - 80
   b. Microbial Studies - 80
   c. Management - 40
2. Facilities - 100
   a. Laboratory space and general equipment available for project - 50
   b. Analytical equipment (GC-MS, etc.) - 50
3. Experience - 100
   a. Environmental Fate Studies - 40
   b. Chemistry and Photochemistry - 30
   c. Microbial Metabolism - 30
4. Personnel - 100

GENERAL SERVICES ADMINISTRATION
FED. PROC. REG. (41 CFR) 1-16.101

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1 1

1. AMENDMENT/MODIFICATION NO.	2. EFFECTIVE DATE	3. REQUISITION/PURCHASE REQUEST NO.	4. PROJECT NO. *(If applicable)*
P-801	77 OCT 25		

5. ISSUED BY CODE
US Army Medical Research
and Development Command
Washington, D.C. 20314

6. ADMINISTERED BY *(If other than block 5)* CODE

7. CONTRACTOR NAME AND ADDRESS *(Street, city, county, state, and ZIP Code)* CODE FACILITY CODE

8.
[X] AMENDMENT OF SOLICITATION NO. DAMD17-78-Q-8390
DATED 77 OCT 24 *(See block 9)*
[ ] MODIFICATION OF CONTRACT/ORDER NO.
DATED *(See block 11)*

9. THIS BLOCK APPLIES ONLY TO AMENDMENTS OF SOLICITATIONS

[X] The above numbered solicitation is amended as set forth in block 12. The hour and date specified for receipt of Offers [ ] is extended, [ ] is not extended.

Offerors must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation, or as amended, by one of the following methods:

(a) By signing and returning 1 copies of this amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKOWLEDGMENT TO BE RECEIVED AT THE ISSUING OFFICE PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If, by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided such telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.

10. ACCOUNTING AND APPROPRIATION DATA *(If required)*

11. THIS BLOCK APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS

(a) [ ] This Change Order is issued pursuant to
The Changes set forth in block 12 are made to the above numbered contract/order.

(b) [ ] The above numbered contract/order is modified to reflect the administrative changes (such as changes in paying office, appropriation data, etc.) set forth in block 12.

(c) [ ] This Supplemental Agreement is entered into pursuant to authority of
It modifies the above numbered contract as set forth in block 12.

12. DESCRIPTION OF AMENDMENT/MODIFICATION

The following is added to Section F Description/Specifications, Paragraph 2a Scope of Work:

The Government estimates that approximately 2.0 man years of professional effort would be required in the conduct of this research.

Except as provided herein, all terms and conditions of the document referenced in block 8, as heretofore changed, remain unchanged and in full force and effect.

13. [ ] CONTRACTOR/OFFEROR IS NOT REQUIRED TO SIGN THIS DOCUMENT [X] CONTRACTOR/OFFEROR IS REQUIRED TO SIGN THIS DOCUMENT AND RETURN ____ COPIES TO ISSUING OFFICE

14. NAME OF CONTRACTOR/OFFEROR	17. UNITED STATES OF AMERICA
BY (Signature of person authorized to sign)	BY (Signature of Contracting Officer)
15. NAME AND TITLE OF SIGNER *(Type or print)* / 16. DATE SIGNED	18. NAME OF CONTRACTING OFFICER *(Type or print)* / 19. DATE SIGNED
	U.S. JEAN V. [illegible] Contracting [illegible] / 26 Oct 77

STANDARD FORM [illegible] 1968
GENERAL SERVICES ADMINISTRATION
FED. PROC. REG. (41 CFR) 1-16.201

REQUEST FOR QUOTATIONS
(THIS IS NOT AN ORDER)

1. REQUEST NO.	2. DATE ISSUED	3. REQUISITION/PURCHASE REQUEST NO.	4. CERTIFIED FOR NATIONAL DEFENSE UNDER BDSA REG. 2 AND/OR DMS REG. 1 RATING:
DAMD17-78-Q-8390	77 OCT 24		DO-C9 (e)

5. ISSUED BY
[illegible] Army Medical Research
[illegible]d Development Command
Washington, D.C. 20314

FOR INFORMATION CALL *(Name and tel. no.) (No collect calls)* Ms. ANN E. MAY AC 202 693-8360

6. DELIVER BY *(Date)* Commence work 30 days after award of contract

7. DELIVERY
☐ FOB DESTINATION
☒ OTHER *(See Schedule)*

8. TO NAME AND ADDRESS
(Street, City, State and ZIP Code)

9. DESTINATION *(Consignee and address including ZIP code)*

10. PLEASE FURNISH QUOTATIONS TO THE ISSUING OFFICE ON OR BEFORE CLOSE OF BUSINESS 21 NOVEMBER 1977 (Date). SUPPLIES ARE OF DOMESTIC ORIGIN UNLESS OTHERWISE INDICATED BY QUOTER. THIS IS A REQUEST FOR INFORMATION, AND QUOTATIONS FURNISHED ARE NOT OFFERS. IF YOU ARE UNABLE TO QUOTE, PLEASE SO INDICATE ON THIS FORM AND RETURN IT. THIS REQUEST DOES NOT COMMIT THE GOVERNMENT TO PAY ANY COSTS INCURRED IN THE PREPARATION OR THE SUBMISSION OF THIS QUOTATION, OR TO PROCURE OR CONTRACT FOR SUPPLIES OR SERVICES.

The following checked sections are contained in the contract.

(X)	Sec.		Page	(X)	Sec.		Page
		PART I - GENERAL INSTRUCTIONS			G	Preservation/Packaging/Packing	
	A	Cover Sheet		X	H	Deliveries or Performance	21
X	B	Contract Form and Representations, Certifications, and Other Statements of Offeror.	3	X	I	Inspection and Acceptance.	21
					J	Special Provisions	
					K	Contract Administration Data	
X	C	Instructions, Conditions, and Notices to Offerers.	11			PART III - GENERAL PROVISIONS	
				X	L	General Provisions	22
	D	Evaluation and Award Factors	15			PART IV - LIST OF DOCUMENTS AND ATTACHMENTS	
		PART II - THE SCHEDULE					
	E	Supplies/Services and Prices		X	M	List of Documents and Attachments	23
X	F	Description/Specifications	18				

ENVELOPES OR WRAPPING SHOULD BE ADDRESSED TO US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND, WASHINGTON, DC 20314 ATTN: SGRD-RM, Ms. ANN E. MAY, RFQ DAMD17-78-C-8390; DELIVERY MAY BE MADE TO ROOM 8F-090, FORRESTAL BUILDING, 1000 INDEPENDENCE AVENUE, S.W., SEE SECTION C, PARAGRAPH 9 ON PAGE 13.

17. PRICES QUOTED INCLUDE APPLICABLE FEDERAL, STATE, AND LOCAL TAXES.

DISCOUNT FOR PROMPT PAYMENT _____ % 10 CALENDAR DAYS; _____ % 20 CALENDAR DAYS; _____ % 30 CALENDAR DAYS; _____ % _____ CALENDAR DAYS.

NOTE: Reverse must also be completed by the quoter.

NAME AND ADDRESS OF QUOTER *(Street, city, county, State, including ZIP Code)*	19. SIGNATURE OF PERSON AUTHORIZED TO SIGN QUOTATION	20. DATE OF QUOTATION
	21. SIGNER'S NAME AND TITLE *(Type or print)*	22. TELEPHONE NO. *(Include area code)*

INFORMATION TO OFFERORS OR QUOTERS

(Section A - Cover Sheet)

SOLICITATION NUMBER [illegible]

[ ] ADVERTISED *(IFB)* [ ] NEGOTIATED *(RFP)*

[X] NEGOTIATED *(RFQ)*

ISSUING OFFICE *(Complete mailing address including Zip Code)*

US Army Medical Research
and Development Command
Washington, D.C. 20314

ITEM(s) TO BE PURCHASED *(Brief description)*

Studies of Environmental Fates of Diisopropyl Methylphosphonate and Decyclopentadiene

THIS PROCUREMENT IS:

[X] UNRESTRICTED [ ] SET-ASIDE *(This is a _____% set-aside for [ ] Small Business, [ ] Labor Surplus Area Concerns or [ ] Combined Small Business/Labor Surplus Area Concerns.) (See Section C of the Table of Contents in this solicitation for details of the set-aside.)*

NOTE THE AFFIRMATIVE ACTION REQUIREMENT OF THE EQUAL OPPORTUNITY CLAUSE WHICH MAY APPLY TO THE CONTRACT RESULTING FROM THIS SOLICITATION.

You are cautioned to note the "Certification of Non-Segregated Facilities" in the solicitation. Failure to agree to the certification will render your reply nonresponsive to the terms of solicitations involving awards of contracts exceeding $10,000 which are not exempt from the provisions of the Equal Opportunity clause.

"Fill-ins" are provided on the face and reverse of Standard Forms 18 and 33, or other solicitation documents and Sections of Table of Contents in this solicitation and should be examined for applicability.

See the paragraph of this solicitation entitled "Late Bids, Modifications of Bids or Withdrawal of Bids" or "Late Proposals Modifications of Proposals and Withdrawals of Proposals".

The envelope used in submitting your reply must be plainly marked with the Solicitation Number, as shown above and the date and local time set forth for bid opening or receipt of proposals in the solicitation document.

If NO RESPONSE is to be submitted, detach this sheet from the solicitation, complete the information requested on reverse, fold, affix postage, and mail. NO ENVELOPE IS NECESSARY.

Replies must set forth full, accurate, and complete information as required by this solicitation *(including attachments)*. The penalty for making false statements is prescribed in 18 U.S.C. 1001.

ADDITIONAL INFORMATION

FOR INFORMATION ON THIS PROCUREMENT WRITE OR CALL

NAME AND ADDRESS	TELEPHONE *(Area Code, No. & Ext.)*	
Ms. ANN E. MAY US Army Medical Research and Development Command, ATTN: SGRD-RM Washington, D.C 20314	(202) 693-8360	NO COLLECT CALLS

5

21 November 1977

A Proposal for Research

STUDIES OF ENVIRONMENTAL FATES OF DIISOPROPYL METHYLPHOSPHONATE AND DICYCLOPENTADIENE

SRI Proposal LSU 77-188

Technical Proposal

Prepared for:

U.S. ARMY MEDICAL RESEARCH AND
DEVELOPMENT COMMAND
Washington, D.C. 20314

Attention: SGRD-RM, Ms. Ann E. May

In response to RFQ DAMD17-78-Q-8390

Submitted by:

Ronald J. Spanggord, Ph.D.
William R. Mabey, Ph.D.
Tsong-Wen Chou, Ph.D.

Approved:

W. A. Skinner, Executive Director
Life Sciences Division

Philip J. O'Donnell, Manager
Contract Administration

333 Ravenswood Avenue, Menlo Park, California 94025

SRI International

SRI International

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INTRODUCTION

Rocky Mountain Arsenal (RMA) has long been used as a facility for the production of chemicals for military use. Over the years, land disposal of such chemicals and the precursors, intermediates, and residues associated with their production has been practiced. The adverse effects of this method of disposal are now becoming apparent: Significant groundwater contamination has been observed beyond the boundaries of the installation. Diisopropyl methylphosphonate (DIMP) and dicyclopentadiene (DCPD) are two compounds that have leached from soils and have reached groundwater supplies.

To understand the environmental impact of such chemicals, their persistence in the environment must be determined and their transformation products that result from known routes of chemical transformation must be identified. Two routes of chemical transformation in the environment are photochemical oxidation and microbial biodegradation. Little information exists about these processes relative to DIMP and DCPD.

The proposed research program is aimed at understanding these processes using laboratory conditions to predict the persistence of DIMP and DCPD and to identify their transformation products resulting from photochemical and microbial processes. Since the USAMRDC has the responsibility of maintaining the environmental quality at military installations, this information will aid it in its decision-making for future studies and pollution abatement practices.

SRI International is pleased to submit this proposal in response to USAMRDC's request.

OBJECTIVES

The objectives of the proposed research are to determine the roles of photolysis and biodegradation in the environmental fate of DIMP and DCPD. This determination will entail the gathering of data on photochemical rates in pure and natural waters, data on microbial degradation rates, and data on complete mineralization in natural waters and soils. Using these data, we will estimate the environmental half-lives for each chemical. Associated with these determinations will be the identification of transformation products in each process and an evaluation of soil activation as a method to accelerate decomposition rates.

BACKGROUND

The effects of environmental contamination are becoming more evident daily, and we are realizing that we must develop a clearer understanding of "where chemicals go."

Over the last two years, SRI International has been actively engaged in this area of research and has developed a computer model system that integrates the experimental data from individual transport and transformation processes to assess the fate of chemicals. Through these studies, we have developed improved methods for measuring kinetics and equilibria in microbial degradation processes, photochemical oxidations in pure and natural waters, solubility, hydrolysis, oxidation in water, volatilization, and sorption partition coefficients onto clay, sediments, and bacteria. Eleven environmental pollutants have been assessed in all these transport and transformation processes.

This experience is highly applicable to the studies outlined in the USAMRDC's RFQ. For the proposed research on DIMP and DCPD, we will develop analytical methodologies and then evaluate photochemical and microbial processes.

METHOD OF APPROACH

Analytical Chemistry

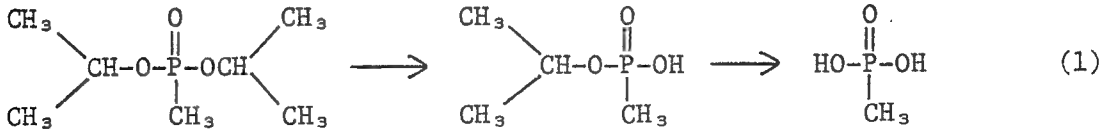
Critical to the photochemical and microbial transformation studies will be the accurate determination of the concentration of the test chemicals at selected times. From this determination, the kinetic data will be derived. The proposed analytical methodologies for DIMP and DCPD are discussed below.

Diisopropyl Methylphosphonate (DIMP)

DIMP will be monitored by gas chromatography (gc) using flame ionization detection. We have evaluated a number of alkyl phosphine oxides and phosphate esters, including trimethyl phosphate, tris(2-aziridinyl)phosphine oxide and tris(2-methylaziridinyl)phosphine oxide, and have found them to be stable under gc conditions. We expect that DIMP will also be amenable to this analytical technique. For added selectivity and sensitivity, we will use an alkali-flame ionization detector that is highly sensitive for phosphorus-containing compounds.

Based on the behavior of alkyl phosphate esters, DIMP should readily partition to an organic solvent (such as ethyl acetate or methylene chloride) for concentration before gc analysis. This should provide a highly sensitive assay system for DIMP.

The transformation products of DIMP will also be analyzed by gc. We expect that hydrolysis products (Eq. 1) may be important in the microbial transformation pathways leading to isopropyl methylphosphonate (IMPA, I) and methylphosphonic acid (MPA, II)..



(1)

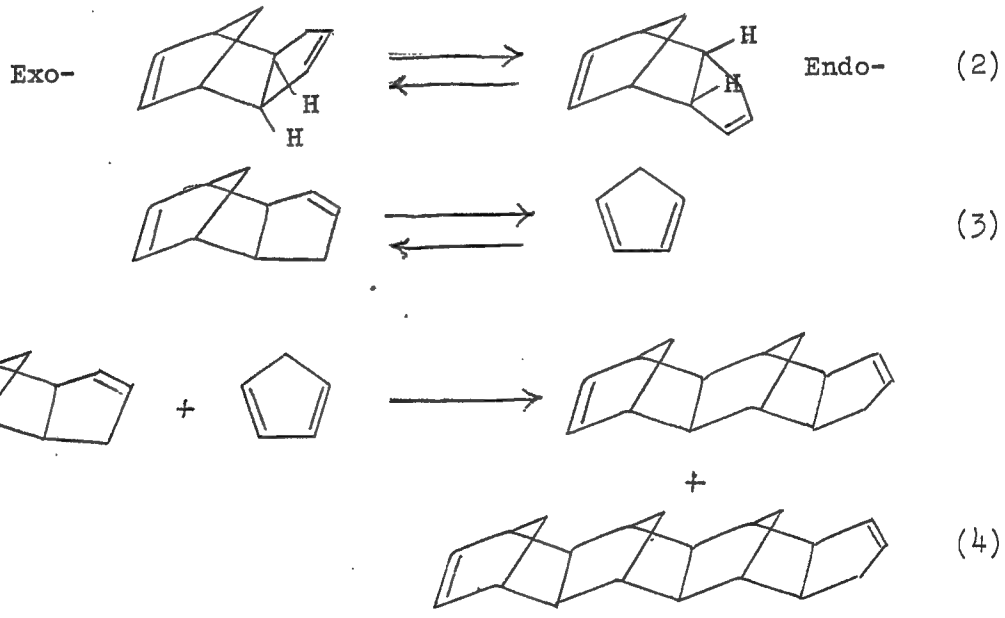
I II

These compounds are not expected to partition readily into an organic solvent as the parent compound does. Analyses for these compounds will be performed by gc on lyophilized residues that have been derivatized with a silylating reagent such as bis-trimethylsilyl)trifluoroacetamide (BSTFA). The products arising in the chromatographic profiles will be identified, and their identity will be confirmed through comparison of their profiles with those of authentic compounds by gas chromatography/mass spectrometry (gc/ms).

Dicyclopentadiene (DCPD)

DCPD will also be monitored by gc using flame ionization detection. Kinkead et al.[1] have shown that this methodology is applicable to DCPD analyses in air.

DCPD may be difficult to obtain in a pure state because of exo-endo equilibration (Eq. 2), dissociation (Eq. 3), and additional reactions to form trimers and tetramers of cyclopentadiene (Eq. 4).



In view of the complexity of these processes, each species must be monitored through the photochemical and microbial transformation processes. For example, exo- and endo-dicyclopentadiene may behave differently in both photochemical and microbial processes or it/they may be interconverted in the transformation process. To monitor a potentially complex mixture, we plan to follow these transformations by capillary gc. In this manner, each specie will be resolved and can be monitored independently from the mixture. This will also aid us in predicting the stereochemistry of transformation products, should one isomer have a significantly greater rate of transformation than another. Again, we will use gc/ms to tentatively identify transformation products, and confirmation will be achieved by comparing their spectral data with those from authentic reference standards.

For studies involving radiolabeled DIMP and DCPD, $^{14}CO_2$ will be trapped in standard Hyamine solutions for direct scintillation counting.

Photochemistry

The following protocol is proposed for the photochemical studies of DIMP and DCPD. This protocol is designed to be responsive to the one outlined in the RFQ, but we have made minor changes in the order of the experiments and in some of the experimental procedures. We also propose to conduct some photolysis experiments in natural waters obtained near RMA. In designing our protocol and approach, we have drawn on experience gained in a current EPA contract (No. 68-03-2227) in which photochemical studies are included in a large interdisciplinary project to measure chemical, physical, and microbial processes determining the fate of organic chemicals in freshwater aquatic systems.

Direct Photolysis Studies

DIMP and DCPD will be photolyzed at concentrations below their solubility limit (or at 1 ppm, whichever is less) in air-saturated pure water. A merry-go-round photochemical reactor with a 450-watt Hg lamp in a Pyrex immersion well will be used. The Pyrex well filters all light below 290 nm and simulates the low-wavelength cutoff of the solar spectrum. In another experiment, we will determine the effect of degassing the reaction solution on the photolysis reaction rate. If a significant photochemical reaction is found by either procedure within 96 hours, the products of direct photolysis will be determined.

The pH of the reaction solutions will be checked before and after photolyses. Our experience in the EPA-supported studies has shown that photolysis of chemicals at concentrations of below 1 ppm in water does not significantly alter the pH of the solution. Dark control

experiments will also be performed to ensure that only photolysis is responsible for loss of chemical.

This procedure is a slight modification of the one outlined in the RFQ. Although we can carry out the photolyses with a 1200-watt Hg lamp for 90 days, as specified in the RFQ, we consider such a procedure unnecessarily long. In studies on the photolysis of p-cresol, we have found a half-life of 16 hours using the 450-watt Hg lamp/Pyrex filter system; in sunlight (mid-spring), p-cresol has a photolysis half-life of longer than 200 days.[2] Although the photolysis rate constant of a specific chemical also depends on its uv absorption spectra and quantum yield, we believe our light source and filter system are adequate for screening for photochemical reactions. We do realize, however, that the Hg lamp/Pyrex (or other borosilicate) glass filter systems do not provide a direct measure of sunlight photolysis rates because the emission lines and relative photon fluxes of the lamp are much different from the continuum of sunlight, which itself changes in relative intensity with the time of day and the time of year.

Note also that we do not expect either DIMP or DCPD to have significant uv absorption above 290 nm, the low-wavelength cutoff of the solar spectrum. Unless either chemical has an absorption that tails into the solar spectrum region, no direct photolysis should occur in the environment. The importance of the absorption tail should not be overlooked, however. In our studies on p-cresol, the absorption tail of p-cresol (with absorption coefficient of 7 $M^{-1}cm^{-1}$ at 300 nm) still gave appreciable sunlight photolysis (the photolysis quantum yield was 0.1 for direct photolysis.)[2]

Direct Photolysis in Buffered Waters

If a photochemical reaction is found in the preceding experiment, we will also conduct photolysis experiments at pH 5 and 9 using the same procedure. Constant pH will be attained by use of solutions of 0.01 M buffer salts; the pH will be checked again at the end of each experiment. If the photolysis rate constant differs from that in the pure water solution by more than 10%, we will perform experiments to determine whether the difference is actually a pH effect or is due to the buffer salts used. The latter effect can be probed by the use of different buffer salt concentrations or use of different buffer salts.

If we determine that the difference in photolysis rate is due to pH, a photolysis experiment will also be carried out at pH 7, with the products determined for all three pH values in both air-saturated and degassed waters. If the photolysis rates in the buffer solutions are the same as those in pure water, we will then conduct photolysis experiments with natural waters, as described below, after a quantum yield has been measured.

Quantum Yield and Calculation of Photolysis Half-Life in Sunlight

If measurable photolysis of either DIMP or DCPD occurs in the preceding experiments, the quantum yield for direct phototransformation of each chemical will be measured at a wavelength agreed upon by SRI and the USAMRDC Project Officer. To calibrate the irradiating light at this wavelength, we will use an o-nitrobenzaldehyde actinometer system.[2]

The uv absorption spectrum of the chemical will also be measured carefully, including any absorption that tails into the solar spectrum region (> 290 nm). Using the uv absorption spectral data and the measured quantum yield, the half-life for direct photolysis will be calculated by the method of Zepp and Cline.[3] The computer program for this calculation is available at SRI and permits calculation of direct photolysis half-lives as a function of time of year and for specific latitudes. Use of this program at EPA-ERL (Athens, Georgia) and at SRI has shown excellent agreement between the calculated half-lives and those measured in sunlight experiments.

Indirect Photolysis Studies

Photolysis experiments on DIMP and DCPD will also be conducted in samples of natural waters obtained in the vicinity of the RMA facility. One water will be from the North Bog, and two other waters may be selected by the Project Officer. If the contaminated groundwater from RMA becomes a surface water, we recommend that it be used in one experiment. We believe that photolysis studies in natural waters are important since literature information as well as our own studies[2] have shown that natural substances in some waters can act as photosensitizers or as free radical photoinitiators and thus accelerate photochemical transformation rates. For instance, in our photolysis studies on p-cresol, we found that 10 ppm humic acid in the p-cresol solution accelerated the phototransformation of p-cresol by a factor of 12 over the rate in pure water.

In the photochemical reaction rate studies in natural waters, we will use the 450-watt Hg light source and merry-go-round photochemical reactor. Initial experiments will be carried out in 0.22-μ filter-sterilized natural waters. Data obtained from these experiments will be compared with the direct photolysis rates for determination of the effect the natural waters have on the photolysis rate in homogeneous soltuion. If biodegradation studies indicate that either or both chemicals are not biodegradable, we may be able to simulate more closely the natural water conditions using autoclaved or untreated natural water samples.

Other Photochemical Studies

As time and funds permit, and after discussion with the Project Officer, we may consider other photochemical studies on DIMP or DCPD. One possible research effort is described below.

The potential of photolytically decontaminating waters using free radical initiators or sensitizers should be considered. Although DIMP is relatively stable in such oxidation reactions,[5] DCPD may be readily degraded in such reactions. For instance, singlet oxygen, which is generated from dye-sensitized photochemical reactions, reacts with olefins such as DCPD.[6] If laboratory studies indicate that such reactions may be feasible for decontamination, the methods might be investigated for use at RMA. Because substances in natural waters are known to produce singlet oxygen,[7] addition of humic/fulvic acids to waters at RMA might also be used to generate singlet oxygen for such oxidations without introducing a "new" or foreign chemical into the environment.

Microbial Studies

General Consideration

To study the biodegradability of organic compounds in a natural environment, a rapid screening test is necessary before an extensive investigation of degradation rates and metabolic products can be performed. This is necessary to determine whether the water or soil systems possess or can develop microorganisms that can degrade chemicals within a reasonable time. When significant degradation of the chemical occurs in the screening test, an enrichment technique can be applied to obtain a mixed population of organisms that are more specific to the biodegradation of the test chemical.

The chemical compositions and the types and concentrations of microorganisms vary with different natural waters and in the same water at different times. Furthermore, only a small portion of microbes present in the water may be involved in the biodegradation of synthetic organic compounds. Therefore, the adaptation period and rate of degradation vary considerably. To circumvent this problem, an acclimated mixed population is used in the study of biodegradation rate when the rate is measured as a function of biomass.

The biodegradations of organic compounds in natural water systems are influenced by many factors, including pH, temperature, oxygen tension, and the nutrients and toxicants present. The pH, temperature, and oxygen tension may vary seasonably, so year-round average values of the parameters are used for the rate studies rather than just one time measurement value.

In nature, water bodies often receive a variety of mixed compounds so the phenomenon of cometabolism can become important.[8] In some cases, an organic substrate may not be degraded unless other growth-promoting organic substrates are present. These growth-promoting chemicals may be analog compounds, or they may be compounds of unrelated structure. An investigation of possible cometabolic degradation may be desirable if DIMP and DCPD are found to be persistent in these studies.

No information on the biodegradation of DIMP has been published. DIMP may be hydrolyzed by microorganisms to IMPA and MPA, although other possible pathways exist. Isopropanol is easily metabolized by microorganisms,[9] but MPA and IMPA may not degrade readily. In fact, these two compounds did not degrade in rat metabolism studies.[10] MPA degradation may be the rate-limiting process in the complete degradation of DIMP.

No literature is available on the biodegradation of DCPD. However, the reported biodegradability of analog compounds such as cyclopentane[11] and heptachlor[12,13] indicates that DCPD may biodegrade.

Sources of Degradation Organisms

The sources of microbial communities for the proposed biodegradation studies are:

- RMA North Bog water with sediment. These samples will be collected and transferred refrigerated to SRI as rapidly as possible.
- Soil samples known to be contaminated with the test chemicals.
- If the samples from RMA North Bog and the soil samples fail to develop acclimated cultures, samples from local eutrophic water bodies or waters with a history of chemical contamination may be used.

Screening for Biodegradation

Water samples with sediment, or soil samples mixed with sterile water, will be mixed well and then allowed to settle. The top water will be used for preliminary degradation screening tests.

The test chemical will be added to 4 to 6 liters of water in a 9-liter glass bottle that has fittings for the introduction of sterile air through ceramic diffusers at the bottom of the bottle, a sampling port, an addition and pressure relief port, and an air exhaust through a sterilizing filter.[14]

We propose that initial screening be performed on solutions containing approximately 10 ppm test chemical. The pH of the water samples will be adjusted and maintained at the pH of the RMA North Bog water. If the data are available, we would prefer to use the year-round average pH of this site.

The bottles will be incubated in a dark constant-temperature incubation room. The temperature for the screening will be the average temperature of the bog site or at an ambient temperature of 20 to 25° C. Sterile air will be slowly introduced into the water through the sparger to maintain aerobic conditions.

An aliquot of water samples will be withdrawn from the bottles periodically, and biodegradation will be monitored by the disappearance of the test chemicals. When significant degradation is observed, an aliquot of the water sample with the developed organisms will be transferred into flasks containing fresh media with basal salts and DIMP or DCPD. Transfer of the organisms to the media may also be done with additional small quantities of metabolizable organic substrate, such as analog compounds or glucose plus yeast extract. If these additional organic substrates are needed for the biodegradation of the test chemicals and if they cannot be eventually eliminated, a cometabolic relationship will be established.

After sequential transfers of the organisms, the enriched mixed population of microorganisms will be preserved under liquid nitrogen storage conditions.

Biodegradation Rate Studies

The biodegradation rate studies will be conducted in stirring fermenters with RMA North Bog water and one of the acclimated mixed cultures established for each test chemical. The water from RMA will be collected, sterilized, and stored under refrigeration before the culture is added.

The pH of the water will be adjusted and maintained at the pH recorded at the site when the sample was collected; either pH buffer or a New Brunswick pH controller with acid or base will be used. The dissolved oxygen level will be maintained at the level of the bog water by aeration with a New Brunswick Dissolved Oxygen controller. If the dissolved oxygen level of the bog water is high, constant aeration will be provided in a fermenter jar or shaker flask.

The temperature used in the test will be that of the bog water, preferably the year-round average temperature. Since seasonal temperature change is great in the area, the use of two temperatures--one of about 25° C and another of about 10° C--is recommended.

Biomass will be monitored by cell count, turbidometry, or ATP analysis. The disappearance of the test chemicals will be analyzed after an aliquot is removed from the fermenter and extracted with solvents. The rate of degradation will be calculated as a function of biomass.

A study may be warranted to investigate the biodegradation of the test chemicals in a basal salts medium instead of in North Bog water. This would circumvent the possible problem of variability of the chemical composition of North Bog water, which may inhibit biodegradation and reproducibility. Moreover, this substitution could reduce the time required for water sampling.

The Rate of Mineralization in Water

For determining the rate of mineralization, radioactive methyl-^{14}C-labeled DIMP or randomly labeled DCPC will be incorporated into the test water, and $^{14}CO_2$ from the fermenter will be trapped in a trapping solution periodically and removed for counting in a scintillation counter. If photolysis of the test chemical has been found to be significant, the overall rate of mineralization will be determined by a combination of the most efficient conditions for photolysis and mineralization. This may involve degradation studies under natural or simulated sunlight conditions.

The Rate of Complete Degradation in Soil

The rate of mineralization in soil will be determined with radiolabeled chemicals incorporated into soil, preferably collected from a site with a history of contamination. The soil with ^{14}C-labeled chemical will be placed in sealed glass containers, CO_2-free sterile air will be slowly introduced onto the surface of the soil, and the produced CO_2 will be trapped in a trapping solution from the exhaust air. The trapping solution will be removed periodically, and radioactivity will be counted in a scintillation counter.

The effect of soil activation will be examined. The soil may be mixed with the broth of an acclimated culture, and the rate of decomposition will be measured. The soil may be enriched by addition of the test chemical with analog compounds and/or glucose and yeast extract. When degradation of the chemicals observed, part of the acclimated soil may be added into new soil with the test chemical to determine the degradation rate.

Determination of Major Biodegradation Products

The major metabolites will be identified from the broth in the fermenters or flasks with test chemical/basal salts media inoculated with enriched cultures. Samples will be withdrawn, extracted with solvents, and analyzed by gc, high-pressure liquid chromatography (hplc), or ^{14}C-labeled compounds will be analyzed by thin-layer chromatography (tlc) using radioautography as a detection technique. This will aid in establishing a material balance for the product distributions observed in the gc or hplc profiles.

STATEMENT OF WORK

SRI Internation will exert its best efforts and supply the necessary personnel, equipment, and facilities to obtain experimental data that will further understanding of the photochemical and microbial transformations of diisopropyl methylphosphonate and dicyclopentadiene. This work will include the determination of rates of photolysis and biodegradation of DIMP and DCPD under laboratory and natural conditions and identification of transformation products.

FACILITIES

General

At SRI International's Menlo Park headquarters, the research facilities comprise more than one million square feet of office and laboratory space and incorporate the most advanced scientific equipment, an extensive library, and specialized support services.

Analytical Studies

The facilities dedicated to the analysis of organic photoproducts and microbial degradation products are extensive, comprising ten laboratories with more than 5000 square feet of space. The major analytical equipment available for this project includes:

- Gas chromatography
  - Varian 2740; FID, AFID, and EC detectors; equipped for capillary operation.
  - Hewlett Packard 5711; two instruments; prep capability.
  - Varian 2100; FID and EC detection capabilities.
- High-pressure liquid chromatography--Spectra-Physics 3500B; three instruments equipped with fixed and variable uv and fluorescence detectors and gradient solvent elution.
- Gas chromatography/mass spectrometry--LKB9000 gc/ms equipped with a PDP-12 computer.
- Nuclear magnetic resonance
  - Varian XL100 equipped with Fourier transform and multinuclear capability.
  - Varian EM-90, A-60A, and T-60.
- Ultraviolet spectrophotometry
  - Cary Models 11, 14, and 15.

- Perkin Elmer 200.

- Thin-layer and paper chromatography--Extensive.

- Counting equipment

  - Mark I liquid scintillation spectrometer (Nuclear Chicago).

  - Autoscanner for paper and thin-layer plates (Vanguard Instrument Corp., Series 880).

  - Liquid scintillation spectrometers, Series 725 (Nuclear Chicago).

  - Tri-Carb sample oxidizer, Model 305.

  - Tri-Carb liquid scintillation spectrometer, Model 314-AK.

Photochemical Studies

The photochemical studies will be performed in approximately 1000 square feet of laboratory space. These laboratories are equipped with a Hanovia 450-watt merry-go-round photochemical reactor, six Hanovia 1200-watt mercury lamps, power supplies, and Pyrex and quartz immersion well reactors that can be used as static or dynamic systems.

Microbiological Studies

For the microbiological studies, approximately 900 square feet of laboratory space will be available for the proposed research. SRI has complete capabilities for growing microorganisms and performing degradation studies. The following major equipment is available for this work:

- Sterile hoods
- Autoclaves
- Walk-in incubator
- Cabinet incubators
- Refrigerated cabinet incubator
- New Brunswick Psycrotherm Incubator shakers
- New Brunswick Incubator shakers
- Double deck rotary shaker
- New Brunswick water bath shaker
- Water bath incubators
- Spectrophotometers
- Aminco ATP photoanalyzer

- Coulter counters
- Phase microscope
- Spinner flask fermenters
- pH and specific ion meters
- Virtis lyophilizer
- Refrigerated and ambient-temperature centrifuges
- New Brunswick automatic colony counter
- Rotary evaporators
- Bioflo bench top chemostats
- Microfern 14-liter fermenters
- New Brunswick pH controller
- New Brunswick dissolved oxygen controller.

RELATED EXPERIENCE OF PROJECT PERSONNEL

This work will be performed under the direction of Dr. Ronald Spanggord, Manager of the Bio-Analytical Chemistry Program in the Life Sciences Division. Dr. Spanggord has had broad experience in conducting studies on environmental fate, photochemical transformation, and microbial biotransformation.

He participated in a two-year study to develop a laboratory model to assess the environmental fate of selected pesticides, phenols, polynuclear aromatic hydrocarbons, and heterocycles for the Environmental Protection Agency (Contract No. 68-03-2227). In this study, rate constants for environmental pollutants were determined for microbial, photochemical, and chemical oxidation and for physical transport processes in fresh water systems. The data were compiled in a computer model to predict environmental half-lives and predominant transformation processes. Dr. Spanggord)s prime responsibility under this contract was to develop the analytical methodologies to follow the transformation of the test chemicals in the microbial studies and to identify transformation products.

Dr. Spanggord has also evaluated the effects of volatilization and photooxidation on 18 components being discharged at trinitrotoluene production facilities (condensate wastewater) for the USAMRDC (Contract DAMD 17-74-L-4115). The components were isomeric mono-, di-, and trinitrotoluenes and isomeric aminodinitrotoluenes. Under the same contract, Dr. Spanggord has been investigating the photochemistry of TNT (pink water) and RDX for product identification, for mammalian and aquatic toxicity studies, and for microbial mutagenesis studies.

Dr. Spanggord also has participated in a project to assess the feasibility of the microbial degradation of surplus pesticides for the USAMRDC (Contract DADA 17-73-C-3124). In this study, degradative rates were established, and products were identified for such compounds as 2,4-D and for numerous analogs of DDT. The results of this work have been published in Applied and Environmental Microbiology.

Dr. William Mabey will be the task leader for the photochemical studies. Dr. Mabey is a Physical Organic Chemist working in the area of environmental chemistry. On an EPA-supported project (Contract No. 68-03-2227) to determine the fate of selected chemicals in freshwater aquatic systems, he was the task leader for the studies of photolysis, oxidation, and hydrolysis. As the project leader of an extension of this EPA project, Dr. Mabey is responsible for writing

and recommending research protocols for measuring these chemical transformation processes for use in environmental fate assessments. Dr. Mabey is also a coauthor of a critical literature review on the hydrolysis of organic compounds in which data are used to estimate hydrolysis half-lives of chemicals under environmental conditions. He has also provided evaluations of the environmental transformation rates and fates of chemicals in the environment for many interdisciplinary projects, including one project for AMRDC (Contract No. DAMD 17-75-C-5071).

The microbial degradation processes will be evaluated under the direction of Dr. Tsong-Wen Chou, Microbiologist and Biochemist. Dr. Chou has been conducting fermentation research for many years, which has involved the production of antibiotics, amino acids, and nucleotides. He also studied the metabolic pathway of microorganisms by applying radioactive compounds. Dr. Chou most recently worked on EPA Contract No. 68-0302227, a determination of the microbiological degradation of selected organic chemical water pollutants. Currently, under an extension of this EPA contract, he is responsible for the evaluation of laboratory tests for microbial transformation of specific water pollutants.

Drs. Spanggord, Mabey, and Chou have worked successfully together as a team in environmental fate studies in the past and together represent the broad range of experience and expertise necessary to perform multidisciplinary research. Their combined efforts will result in a fluent execution of the proposed work and a detailed understanding of the processes involved in assessing the environmental fate of DIMP and DCPD.

PROJECT ORGANIZATION

The organization of this project will be based on the close interaction of members from three groups--analytical chemistry, physical organic chemistry, and microbiology. Figure 1 depicts this organization.

Dr. Ronald Spanggord will be directly responsible for the timely performance of the project and for communications with the Project Officer. He will devote 20% time to the project.

Drs. William Mabey (10% time) and Tsong-Wen Chou (30% time) will be responsible for investigating the photochemical and microbial transformation processes, respectively. Dr. Spanggord will interact with both groups to provide analytical chemistry support and to make product identifications. Dr. Nestor Bohonos will be available for consultation on the microbial degradation studies. Biographies of Drs. Spanggord, Mabey, Chou, and Bohonos are appended.

Table 1 is the personnel utilization chart indicating the duties of the personnel and distribution of manpower estimated for this project. Figure 2 shows the projected performance schedule for the various tasks outlined in this proposal.

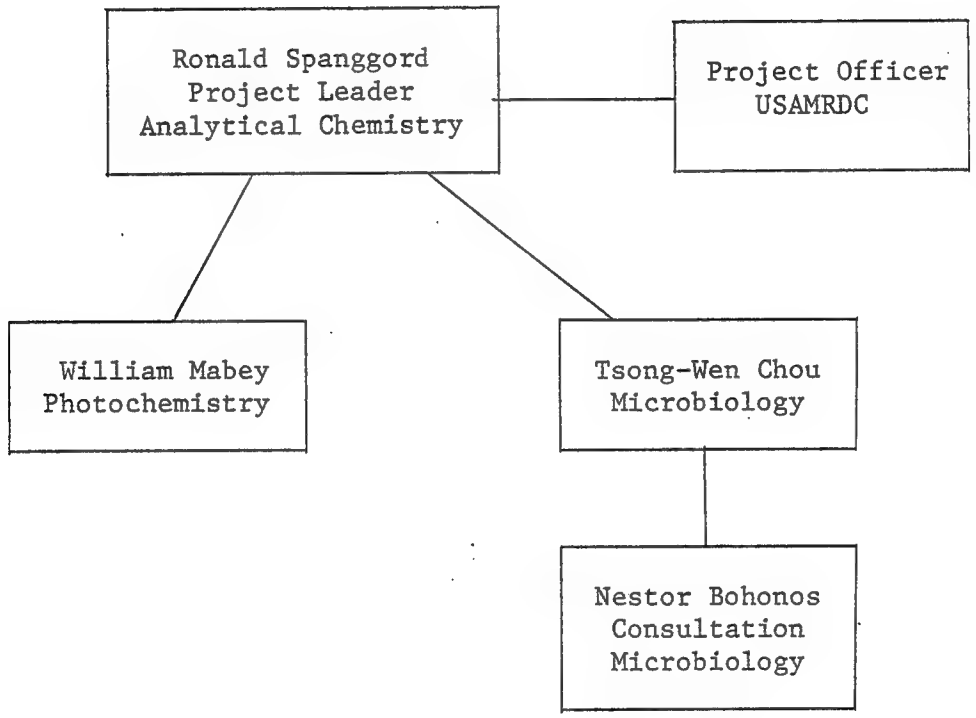


FIGURE 1 PROJECT ORGANIZATION

Table 1

PERSONNEL UTILIZATION

Employee	Function	Discipline	Highest Degree	Task	Planned Hours
Ronald Spanggord	Analytical-Organic Chemistry	Organic Chemistry	Ph.D.	Analysis and Product identefication	370
William Mabey	Photochemistry	Physical Organic Chemistry	Ph.D.	Photochemical Transformation	185
Tsong-Wen Chou	Microbiology	Microbiology	Ph.D.	Microbial Transformation	550
Claire Ingersoll	Chemical Analysis	Organic Chemistry	B.S.	Chemical Analysis	740
Bosco Lan	Photochemistry	Physical Chemistry	M.S.	Photochemical Studies	462
Biological Technician	Microbiology	Biology	B.A.	Microbial Transformation	1,480
Nestor Bohonos	Consultation	Microbiology	Ph.D.	Microbial Transformation	90

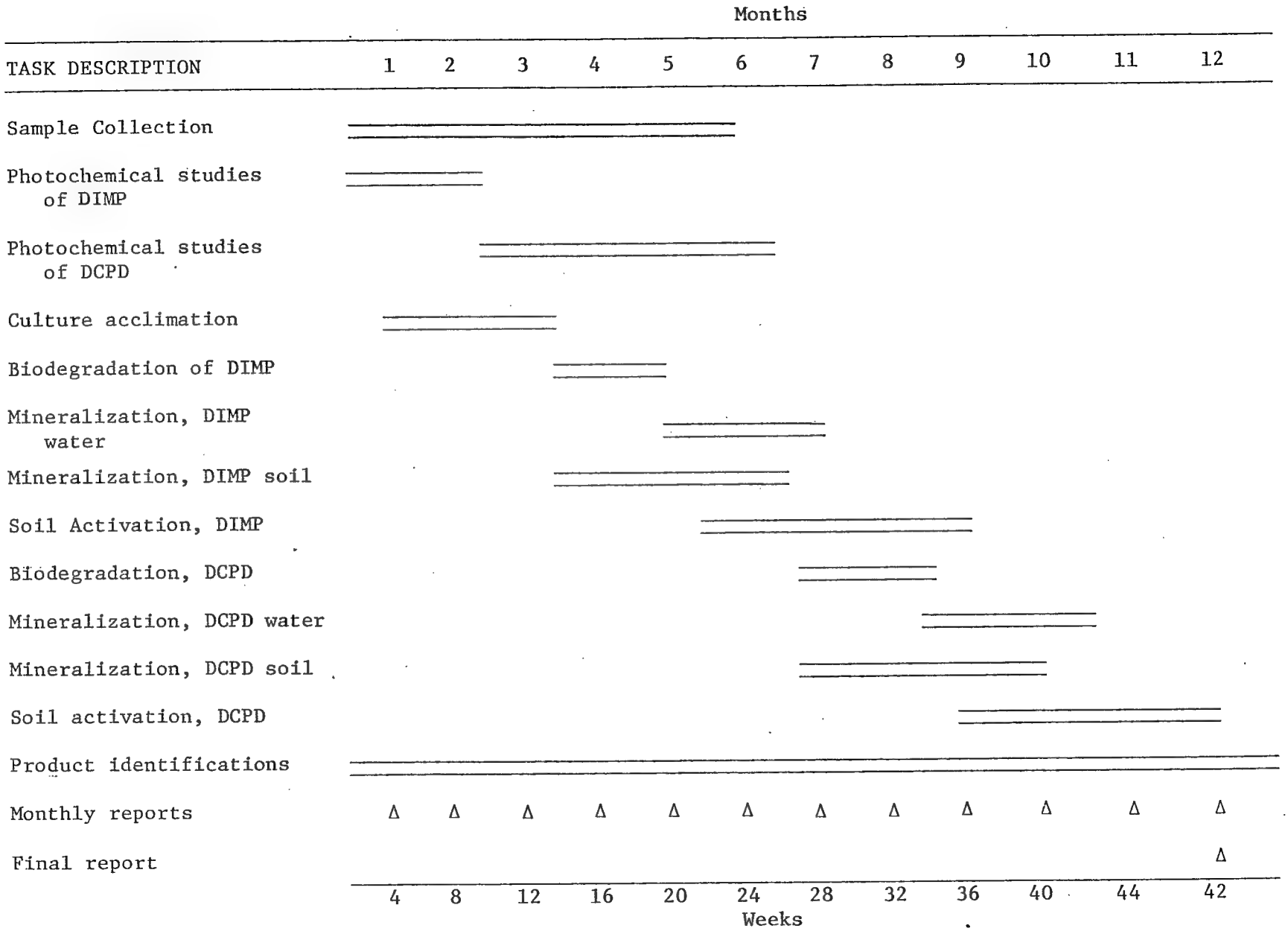


FIGURE 2 PERFORMANCE SCHEDULE FOR PROJECT TASKS

SRI QUALIFICATIONS AND RELATED EXPERIENCE

General

SRI International is an independent research organization owned and governed by its Board of Directors. There is no formal operational connection between nearby Stanford University and SRI, but active professional cooperation is maintained in research, educational activities, and other objectives of mutual interest. SRI is chartered as a nonprofit corporation in the State of California, and no part of its net earnings may inure to the benefit of any private individual.

The basic purpose of SRI is to serve, on a self-sustaining but nonprofit basis, the research needs of industry and government. This is accomplished through specific research projects for individual clients; these projects are SRI's primary activity and source of income.

The Institute currently has a staff of 3,146 people, of whom 1,727 are professionals. Advanced degrees are held by over 1,149 staff members; of those, 529 hold doctorates.

Research activities are conducted in seven discipline-oriented divisions--urban and social systems, management and economics, physical sciences, life sciences, electronics and radio sciences, information sciences, and engineering systems--and in eight topic-oriented centers--resource and environmental systems, minerals and metals, energy, chemical industries, transporation, occupational and environmental safety and health, strategic studies, and economic policy. With a staff of over 3000, representing over 100 disciplines, SRI is preeminent among research organizations in its ability to solve interdisciplinary problems.

Life Sciences Division

The Life Sciences Division at SRI encompasses more than 60 research programs utilizing both multidisciplinary and unidisciplinary approaches. The staff totals about 295, including 81 with Doctorates, 32 with Master's degrees, and 100 with Bachelor's degrees. Disciplines represented in the scientific staff include pharmacology, toxicology, pathology, histology, electron microscopy, experimental psychology, general physiology, neurophysiology, food sciences and nutrition, plant sciences, basic biology, virology, aquatic and marine biology, immunology, parasitology, microbiology, biochemistry, and organic chemistry.

The majority of the projects in Life Sciences are contracted with government agencies, such as the National Institutes of Health, Food and Drug Administration, Environmental Protection Agency, National Aeronautics and Space Administration, Department of Agriculture, and various branches of the Department of Defense. Industrial support comes from numerous commercial companies.

Physical Organic Chemistry Group

The Physical Organic Chemistry Group of the Physical Sciences Division has extensive experience in determining knowledge of reaction mechanisms and kinetics in solution and the gas phase. These include kinetic and product studies on free radical, ionic, and photolytic reactions related to environmental transformation processes.

Related Projects

SRI recently completed a two-year project for the Environmental Protection Agency (Contract No. 68-03-2227) in which a methodology was developed for determining the fate of organic chemicals in freshwater ecosystems.* Our approach was to use experimental data on individual transport and transformation processes and integrate them using computer model simulations to assess the fate of chemicals. For this study, SRI researchers developed improved methods for measuring the kinetics and equilibrium of the following processes:

- Volatilization from water.
- Solubility in water.
- Sorption partition coefficients onto a clay, several natural sediments, and a mixture of bacteria.
- Photolysis in pure and natural water.
- Hydrolysis in pure and natural water.
- Oxidation in water.
- Degradation by aquatic bacteria.

In an extension of this contract, SRI is critically reviewing the methods described in the literature that have been used to study these processes. We are preparing and recommending detailed protocols for measuring these processes for use in quantitative environmental assessments.

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*Environmental Pathways of Chemicals in Freshwater Systems. Part I. Background and Experimental Procedures. In press (EPA-600/7-77-113, October , 1977). The final draft of Part II, Laboratory Studies, has been submitted to the EPA and contains the experimental data on and assessment of 11 organic chemicals that were studied.

Other contracts related to the proposal work are listed below:

- Mammalian Toxicological Evaluations of TNT Wastewater (Pink Water); Contract DAMD 17-74-C-4115, U.S. Army Medical Research and Development Command

  In this study, synthetic and authentic TNT wastewaters have been photolyzed to several TNT degradation levels under variable pH, concentrated by lyophilization, profiled by hplc, glc, and tlc techniques, and evaluated for acute oral toxicities in mice.

- Toxicity of TNT Wastewater (Pink Water) to Aquatic Organisms; Contract DAMD 17-75-C-5056, U.S. Army Medical Research and Development Command

  Under this contract, the toxicity of various synthetic and authentic TNT wastewaters (see above contract) were evaluated in aquatic organisms.

- Microbiological Decomposition of Standard Military Pesticide Formulations; Contract No. DADA 17-73-C-3124, U.S. Army

  Under this contract, the Pharmaceutical Analysis Department has been measuring rates of degradation of pesticides and herbicides subjected to microbiological systems. An important aspect of this program is to identify metabolites and monitor their rates of formation and degradation. Chemicals investigated in this program include DDT, dieldrin, chlordane, lindane, 2,4-D, and 2,4,5,-T.

- Biological Hazards of Phototransformed Pesticides; Contract R801-840-03, Environmental Protection Agency

  Under this contract, pesticides are exposed to ultraviolet light, sunlight, and photochemically generated singlet oxygen. The reaction products are then isolated, characterized, and submitted for toxicological evaluation.

- Synthesis of Ring A Bi- and Tricyclo Steroids with Potential Antifertility Activity; Contract NO1-HO-3-2741, National Institute of Child Health and Human Development

  Under this contract, ring-A tricyclosteroids are prepared by the photochemical cyclo addition of vinyl olefin functions to 3-keto-Δ^4-steroids. These products are isolated, characterized, and investigated for their potential antifertility activity.

- Synthesis and Analytical Studies in Anticancer Agents; Contract NIH-71-2070, National Institutes of Health

  Under this contract, preparative-scale photochemical reactions have been used in the synthesis of compounds with potential anticancer activity. These reactions have involved the photocyclization of cis-stilbene derivatives to yield polycyclic compounds.

- Analysis of Organic Chemicals Products. Investigation of New Drugs and Composition Thereof; Contract No. DADA-17-73-C-3171, U.S. Army Medical Research and Development Command

  For the past six years, we have been analyzing antimalarial and antiradiation compounds. During this period, additional experience has been gained in analyzing a wide variety of alkaloids, halophenanthrene-methanols, thiophosphoric acids, and aminoarylsulfones as bulk chemicals and formulations. As with the analysis of antitumor compounds, the development of chromatography procedures and the purification of materials for reference purposes were also necessary. While analyzing the impurities found in commercial diaminodiphenylsulfone, a pressure-liquid-column chromatographic procedure was adapted. An example of our proficiency in isolation and identification of minor contaminants is illustrated in our work with commercial dihydroquinine (J. Org. Chem. 33, 3005, 1968).

- Study of Combined Effects of Chemical Carcinogens and Other Chemicals; Contract No. NCI-E-C-71-17, National Cancer Institute, NIH

  Under this contract, the Pharmaceutical Analysis Department is responsible for the procurement, purification, identification, and quantitation of chemical carcinogens, and environmental materials such as nitrilotriacetic acid and several pesticides. These chemicals are incorporated in animal feeds, and the distribution of these tests chemicals and their stability in feeds are determined. In the course of this work, methods were devised to separate, identify, quantitate, and detoxify these test chemicals.

- Hydrolysis of Organic Compounds under Environmental Conditions; Contract No. 5-35905, Department of Commerce

  In this critical review, we are examining the rate constants for hydrolysis in water of 12 classes of more than 200 organic compounds. The objective of using these data is to estimate the persistence of these compounds in freshwater aquatic systems. Primary data were obtained in a critical literature review. Environmentally relevant hydrolysis rate constants and half-lives were then estimated for the conditions of pH 7 and 20° C.

- Chemical Oxidation Processes in Aquatic Environments; Contract No. ENV76-11153, National Science Foundation

  This study is designed to identify important oxidizing species responsible for transformations of organic compounds in aquatic environments and to investigate factors that control oxidant concentrations and reactivity in these environments. The results of this study are being used to elucidate the chemistry occurring in aquatic environments, and they will permit determination of what laboratory experiments can best be used to realistically estimate the persistence of organic compounds under environmental conditions.

- Oxidation of Sulfur and Amine Compounds, Contract No. DAAA15-74-C-0150, Edgewood Arsenal, U.S. Army

  Studies were directed toward developing more rapid methods of detoxification of hazardous sulfides and amines using oxygen, peroxides, and photooxidation.

- Micellar Catalysis - A Method for Detection of G Agents; Contract No. DAAA15-74-C-0516, Edgewood Arsenal U.S. Army

  New techniques were developed to speed hydrolytic detoxivication of fluorophosphate esters.

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APPENDIX - BIOGRAPHIES

RONALD J. SPANGGORD

Manager, Bioanalytical Chemistry Program
Pharmaceutical Analysis Department
Life Sciences Division

SPECIALIZED PROFESSIONAL COMPETENCE
- Qualitative and quantitative analysis of organic compounds in water, feed, and biological systems
- Analysis of carcinogens, pesticides, and environmental chemicals in animal feeds

REPRESENTATIVE RESEARCH ASSIGNMENTS AT SRI (1966-67; 1971-present)
- Analysis of products in aqueous ozone and chlorine dioxide treatments
- Development of methods for the assay of selected chemical carcinogens that have been incorporated into animal feed
- Analysis of pesticide degradation products and the biotransformation of pollutants in microbial systems
- Analysis and identification of TNT photolytic products in wastewaters
- Analytical characterization of new artificial sweeteners
- Synthesis and analysis of chemical carcinogens
- Postdoctoral fellow (1971-72)
- Organic chemist (1966-67)

OTHER PROFESSIONAL EXPERIENCE
- Lecturer in chemistry, San Francisco State College (1968)
- Research assistant, University of Arizona (1969-71): synthesis and identification of amino-organomercurials and synthesis of bicyclic derivatives

ACADEMIC BACKGROUND
- B.S. (1965) and M.S. (1968) in chemistry, San Francisco State College; Ph.D. in chemistry (1971), University of Arizona

PUBLICATIONS
- Coauthor of six technical publications

PROFESSIONAL ASSOCIATIONS AND HONORS
- American Chemical Society
- Alpha Chi Sigma

April 1977

RONALD J. SPANGGORD - SELECTED PUBLICATIONS

H. K. Hall, Jr., J. P. Schaefer, and R. J. Spanggord. Aminomercuration of olefins. J. Org. Chem. 37, 3069 (1972).

R. J. Spanggord, O. K. Chee, D. Z. Dalomeque, and P. Lim. GLC analysis of dialkylnitrosaminos in feed studies. Presented at the 86th Meeting of the AOAC, Washington, D.C., October 1972.

A. J. Francis, R. J. Spanggord, and G. I. Ouchi. Degradation of lindane by Escherichia coli. Appl. Microbiol. 29, 567 (1975).

Tsong-Wen Chou, S. S. Lee, R. J. Spanggord, and N. Bohonos. Continuous-flow compartmentalized fermentation units in environmental degradation studies. Presented at the 172nd American Chemical Society National Meeting, San Francisco, California, August 1976.

T. W. Chou, R. J. Spanggord, E. Shingai, and N. Bohonos. The fate of methyl parathion in freshwater systems. Presented at the 172nd American Chemical Society National Meeting, San Francisco, California, August 1976.

A. J. Francis, R. J. Spanggord, G. I. Ouchi, R. Bramhall, and N. Bohonos. Metabolism of DDT analogues by a Pseudomonas sp. Appl. and Environ. Microbiol. 32, 213 (1976).

R. J. Spanggord and V. J. McClurg. Ozone methods and ozone chemistry of selected organics in water. Proceedings from the International Ozone Institute, Cincinnati, Ohio, 1976.

R. J. Spanggord, A. J. Francis, G. I. Ouchi, and N. Bohonos. Cometabolism of DDT analogues by a Pseudomonas sp. Appl. and Environ. Microbiol. To be published in February issue, 1978.

J. A. Cetruvo, V. F. Simmon, and R. S. Spanggord. Investigation of Mutagenic Effects of Products of Ozonation Reactions in Water. Annals of the New York Academy of Sciences, Vol. 298, 124 (1977).

WILLIAM R. MABEY

Physical Organic Chemist
Physical Sciences Division

SPECIALIZED PROFESSIONAL COMPETENCE

Chemical kinetics; environmental chemistry; mechanisms of hydrolysis, oxidation, and photochemical reactions; free radical chemistry; estimations of degradation kinetics and fate of chemicals in environment

REPRESENTATIVE RESEARCH ASSIGNMENTS AT SRI (since 1972)

Critical review on hydrolysis rates of organic compounds applied to conditions of aquatic environment

Kinetics of gas-phase hydroperoxyl radical reactions in atmosphere

Kinetics of oxidation, hydrolysis, and photochemical reactions of organic pollutants in aquatic environments

Estimation of degradation rates and fates of chemicals in the environment for assessments of hazard and research priority

ACADEMIC BACKGROUND

B.A. in chemistry (1965), University of California (Riverside); M.S. in organic chemistry (1968), California State University (San Diego); Ph.D. in organic chemistry (1972), University of Oregon

PUBLICATIONS

Six publications in physical organic chemistry

PROFESSIONAL ASSOCIATIONS

American Association for the Advancement of Science
American Chemical Society

LANGUAGE PROFICIENCY

Reads scientific literature in German and French

September 1977

WILLIAM R. MABEY, SELECTED PUBLICATIONS

Mabey, W., and T. Mill. Kinetics of hydrolysis and oxidation of organic pollutants in the aquatic environment. Presented at the Symposium on Nonbiological Transport and Transformation of Pollutants on Land and Water: Processes and Critical Data Required for Predictive Description. May 11-13, 1976, National Bureau of Standards, Gaithersburg, Md.

Mabey, W., A. Baraze, B. Lan, H. Richardson, D. G. Hendry, and T. Mill. The fate of methyl parathion in freshwater systems: II. hydrolysis and photolysis. 172nd American Chemical Socity National Meeting, San Francisco, September 1976, Pesticide Division, p. 61.

Mabey, W., and T. Mill. Critical review of hydrolysis of organic compounds in water under environmental conditions, In press (J. Phys. Chem. Ref. Data, 7, 000 (1978).

TSONG-WEN CHOU

Microbiologist-Biochemist
Pioneering Research
Life Sciences Division

SPECIALIZED PROFESSIONAL COMPETENCE

Biochemistry, microbiology, fermentations, food science and technology

REPRESENTATIVE RESEARCH ASSIGNMENTS AT SRI

Research in applied microbiology; daunorubicin fermentation; biodegradation of miscellaneous pollutants utilizing enrichment and cometabolic procedures; fermentation kinetics and biosorptions

OTHER PROFESSIONAL EXPERIENCE

Senior microbiologist, supervisor, Microbiology Laboratory, Rachelle Laboratories, Inc: strain improvement and optimization of tetracycline fermentation process at laboratory and pilot-plant scale; exploratory development work on bacitracin and lysine fermentations; biotransformation of tetracyclines

Research biochemist, University of California at Davis: study of metabolic pathway of ethylene biosynthesis by a mold

Research associate, Massachusetts Institute of Technology: single-cell protein research, especially on thermophilic hydrocarbon fermentation; microbial physiology and isolation characterization of metabolic products

Research assistant, Utah State University: physiological and biochemical effects on mold of gamma radiation

Research associate, Wei-Chuan Foods Company: research on fermentation production of glutamic acid; fermentative degradation of RNA to nucleotides; research on strain selection, optimum fermentation conditions and isolation purification of amino acids and nucleotides

ACADEMIC BACKGROUND

B.S. (1955) in agricultural chemistry, National Taiwan University; Ph.D. (1969) in food sciences and technology (food microbiology and biochemistry), Utah State University

PUBLICATIONS

Author of ten publications

PROFESSIONAL ASSOCIATIONS AND HONORS

American Chemical Society; American Society for Microbiologists; Institute of Food Technologists

Sigma Xi

August 1977

TSONG-WEN CHOU, SELECTED PUBLICATIONS

Studies on production of nucleic acid derivatives by microorganism. I. Isolation of microorganism degrading RNA into 5' nucleotides from the soil of Taiwan area. Ueda et al. J. Chinese Agr. Chem. Socie. 2:44 (1964).

Studies on production of nucleic acid derivatives by microorganism. II. Studies on the optimum condition of degrading RNA into 5' nucleotides with the culture filtrate of Streptomyces No. 8:(1). Lu, Y. Y. et al. J. Chinese Agr. Chem. Socie. 2:50 (1964).

Influence of phosphate compounds on certain fungi and their preservative effects on fresh cherry fruit (Prunes cerasus, L.). Post, F. J. et al. Appl. Microbiol. 16:138 (1969).

Effect of gamma radiation on Penicillium expansum L. I. Some factors influencing the sensitivity of the fungus. Chou, T. W. et al. Radiation Botany. 10:511 (1970).

Effect of gamma radiation on Penicillium expansum L. II. Some enzymatic changesin the fungus. Chou, T. W. et al. Radiation Botany 10:517 (1970).

Effect of gamma radiation on Penicillium expansum L. III. On nucleic acid metabolism. Chou, T. W., D. K. Salunkhe, and B. Singh. Radiation Botany. LL:329 (1971).

Production of an autoinhibitor by a thermophylic Bacillus. Chou, T. W., R. Greasham, S. R. Tannenbaum and A. L. Demain. J. Bacteriol. 111:459 (1972).

The biogenesis of ethylene in Penicillium digitatum. Chou, T. W. and S. F. Yang. Arch. Biochem. Biophy. 157:72 (1973).

Continuous-flow compartmentalized fermentation units in environmental degradation studies. Chou, T. W., S. S. Lee, R. J. Spanggord, N. Bohonos. ACS National Meeting (abstract) (1976).

The fate of methylparathion in freshwater systems: IV. Biodegradation tudies. Chou, T. W., R. Spanggord, E. Shingai, and N. Bohonos. ACS National Meeting (abstract) (1976).

NESTOR BOHONOS

Staff Scientist
Pioneering Research Department
Life Sciences Division

SPECIALIZED PROFESSIONAL COMPETENCE
Biochemistry, microbiology, virology, immunology, and oncology

REPRESENTATIVE RESEARCH ASSIGNMENTS AT SRI (since 1973)
Directing research on in vitro and in vivo antimicrobial evaluations and on microbial decomposition of herbicides and pesticides
Mutagenicity testing with bacteria, yeasts, and animal cell cultures
Studies with biologically active products and microbial processes and of biodegradations in the environment
Determination of high pressure effects on microorganisms

OTHER PROFESSIONAL EXPERIENCE
Chief, Laboratories of Biochemistry, Children's Cancer Research Foundation: research on characteristics and prevention of immune-complex destruction of blood platelets, the serotonin metabolism of platelets, and a cell division factor in synchronously cultivated algae and yeasts
Associate in pathology, Harvard Medical School
Associate director, Chemotherapy Research Section; head, Fermentation Biochemistry Research Department; and head, Mycology Research Department, Lederle Laboratories: microbial product research program involving the discovery, fermentation, isolation, determination of structure, and biological evaluation of numerous antibiotics, including tetracycline, puromycin, profiromycin, alazopeptin; steroid transformation products such as triamcinolone, alkaloid transformations and miscellaneous pharmacologically active agents of microbial origin
Assistant professor and assistant chemist, Department of Agricultural Chemistry, Purdue University; and consultant, Pitman Moore Co.
Research scientist, the Upjohn Co.: responsible for development of submerged penicillin fermentation process and new antibiotic research program
Research scientist, Lederle Laboratories: isolation of biotin, folic acid, aspergillic acid, clavacin, citrinin, pernicious anemia factor, antishock factor; organic syntheses of hydroxynaphthoic acids, pyridazine carboxylic acids, and pterins

ACADEMIC BACKGROUND
B.Sc. in chemistry (magna cum laude, 1937), University of Alberta (Edmonton); M.S. (1939) and Ph.D. (1943) in biochemistry, University of Wisconsin

PUBLICATIONS AND PATENTS
Author or coauthor of more than 60 publications
Six patents

NESTOR BOHONOS (concluded)

PROFESSIONAL ASSOCIATIONS AND HONORS

American Chemical Society (chairman, Fermentation Subdivision, Division of Agricultural Food and Chemistry); American Society for Microbiology; American Society of Biological Scientists; New York Academy of Science (fellow); Society for Industrial Microbiology; editorial boards of Antimicrobial Agents and Chemotherapy, Applied Microbiology, and Journal of Antibiotics; Gamma Alpha; Phi Lambda Upsilon; Sigma Xi

AMENDMENTS

Amendment to Proposal LSU 77-188
"Studies of Environmental Fates of Diisopropyl Methylphosphonate and Dicyclopentadiene"

This amendment is our disposition to the revisions and/or clarifications requested by the U.S. Army Medical Research and Development Command under RFQ DAMD 17-78-G-8390 and should be incorporated as part of our proposal LSU 77-188 entitled "Studies of Environmental Fates of Diisopropyl Methylphosphonate and Dicyclopentadiene." The references submitted in this amendment are intended to supplement those listed on page 29 or our original proposal.

Our response to the points described in the letter of 22 March to Mr. L. E. Tischler, SRI Contracts Administrator, from Ms. Jean V. Smith, USAMRDL Contracting Office, are listed below.

a.1. Our proposal already specifies the use of the merry-go-round (MGR) photochemical reactor (page 6). This apparatus was purchased from Ace Glass, and has been equipped and modified for use in previous environmental studies. MGR reactors are essential for even irradiation of samples in photochemical kinetic measurements. The MGR sample holder can hold 33 sample tubes containing approximately 4 ml each. The tubes are usually tightly stoppered, but can be sealed if volatilization of DCPD or DIMP is found to be a serious problem. All equipment and glassware, including the photochemical reaction tubes, are routinely sterilized in an autoclave or 500° C oven before use to avoid biotransformation processes. The solutions used in photolyses are usually air-saturated, and will provide sufficient oxygen for photooxidations when concentrations of DCPD or DIMP are less than 1 ppm. Sampling time intervals will be determined by the rate at which the chemicals photolyze, with samples taken to beyond two half-lives to verify first order kinetic law behavior during photolysis.

a.2. Our proposal already includes a discussion and description of work for photolyses of DCPD and DIMP in natural waters (page 8 and 9, Indirect Photolysis Studies and Other Photochemical Studies). As stated in our proposal, natural waters have been routinely sterilized in previous studies by filtration through 0.22μ Millipore filters; the filtration also provides homogeneous solutions which are necessary for conducting meaningful solution kinetic studies. We consider filtration-sterilization a preferrable technique since other sterilization methods may alter the sensitizers present or add new chemical sterilants to the natural water, and thereby may change the photochemical properties of the water. As stated

on page 8, if either DCPD or DIMP are found to be resistant to biotransformation, we may be able to conduct some photolyses in untreated natural waters to measure what effect the heterogeneous components have on the photolysis rates. Dark controls reactions are also routinely maintained on all photochemical experiments to identify adventitious processes such as biodegradation or volatilization.

a.3. Regarding the effect of pH on the photolysis rates of DCPD and DIMP, our proposal approached the direct photolysis studies in two stages. The first stage was to conduct photolysis in pure water to determine if any photochemical reaction occurred (pages 6 and 7). As stated in the last paragraph of Direct Photolysis Studies (on page 7), we do not expect direct photolysis of DIMP or DCPD to be an important process in the environment because their absorption coefficients beyond 290 nm are expected to be very small. If a photochemical reaction was found, however, a second stage of experiments in buffered waters was planned (page 7, Direct Photolysis in Buffered Waters).

The criteria as described in item a.(3) of the Request for Quotation, Revisions, is probably applicable to studies on DIMP. This criteria assumes that if no differences are found in the uv absorption spectra of DIMP in solutions of different pH's (5, 7, and 9 as per the RFQ), then the photochemical rates and products are independent of pH. While exceptions to this criteria can be argued, they are difficult to document, and we accept the criteria as stated.

We believe, however, that the procedure cannot be applied to DCPD, since its very low solubility in water and small uv absorption coefficients will make any measurement of a uv spectrum in aqueous solution very difficult, and therefore preclude any pH dependence studies. For direct photolysis studies on DCPD we would then prefer to proceed as described in our proposal.

We also point out that the photolyses in pure unbuffered water are necessary, at least as a control for photolyses in natural waters, so that the direct and sensitized photolyses can be compared.

a.4. If loss of either DCPD or DIMP is found in dark control experiments, we will necessarily identify both the loss process and products, and take precautions against such adventitious processes whenever possible.*

*With regard to the problem of biodegradation during photolysis studies, some experiments at SRI with p-cresol in water found that the dark control tubes showed more rapid loss of cresol than the tubes which were photolyzed. The light apparently killed the organisms responsible for biotransformation. Good photochemical kinetics were obtained and no loss of cresol in the control tubes was found when the water and glassware were sterilized. These experiments suggest that simply subtracting a contribution from biotransformation from an observed photolysis rate will not provide a reliable "corrected" photolysis rate.

Since we expect both DIMP and DCPD to be inert to chemical hydrolysis and oxidation under the control reaction conditions, volatilization and/or biotransformation would be the processes most suspect. If biotransformation is found, we will coordinate our product identification studies with the microbiological studies to avoid repetition of effort.

a.5. SRI recognizes that "negative results", i.e., no reaction, may be a result of some studies, and that such conclusions require as much experimental description and documentation as do positive results and kinetic data. All photochemical experiments described in our proposal are environmentally relevant, and we do not consider any to constitute forcing conditions. Although photochemical studies in natural waters were not included in the original RFQ, we included such studies in our proposal since our previous studies indicate that substances present in natural waters can substantially promote or retard photolysis rates of chemicals when compared to those in pure water solutions. Studies offered in our proposal under Other Photochemical Studies were intended only as possible decontamination methods, and will be carried out only after discussions and written approval of the USAMRDC Project Officer.

b.1. The use of mixed cultures was stated in our proposal under Biodegradation Rate Studies, page 11. The use of mixed cultures is more representative of environmental conditions and allows the opportunity for other organisms to utilize toxic products which may build up from a primary biodegradation step.

b.2. Our proposal stated (page 12, paragraph 1) that the rate of degradation will be determined by the disappearance of the test chemicals. This will be monitored by gc analysis of the parent compound. On the same page, under The Rate of Mineralization in Water, we stated that the rate of mineralization will be determined by $^{14}CO_2$ evolution and not by the disappearance of the parent compound.

b.3. The toxic level of the test chemicals will be determined using a natural mixed population of soil and pond microorganisms. The source of the organisms will be from the Rocky Mountain Arsenal area. The soil and water will be added to a liquid growth medium and incubated in a shaker flask. The growth media will then be used to inoculate duplicate media at different levels of the test compounds (0 to 50 μg/ml). The growth of the microorganisms will be monitored by agar plate count and growth inhibition will be measured. From these data, concentrations of test chemicals below toxic levels can be established for the kinetic experiments.

b.4. Our proposal recommended the use of two temperatures to perform the rate studies (page 11, paragraph 7). The temperatures recommended were 25° C and 10° C; however, these temperatures can be modified according to actual temperatures observed at the sampling site(s).

b.5. The soil minerilization experiment will be performed according to the method of Kazano, et. al.[1] DCPD will be applied to the soil in small quantities of acetone which will readily evaporate. DIMP will be applied to the soil from an aqueous solution since this compound has appreciable water solubility.

For $^{14}CO_2$ evolution studies, metabolic flasks as described by Barth and Pramer[2] will be used instead of the air-flowing containers as originally described.

For the soil activation study, a soil perfusion system as described by Kaufman[3] will be used to obtain enrichment cultures which will then be added to the soil for rate studies.

b.6. All microbiological work is performed under aseptic conditions as a matter of good laboratory practice. Samples will be collected aseptically in autoclaved pyrex glass bottles and transported to SRI as quickly as possible. All equipment and glassware will be autoclaved and chemicals or added buffer solutions will be membrane filtered or dissolved in organic solvents which will be self-sterilizing. As mentioned on page 10, paragraph 6, all air being used in these studies will be drawn through sterilizing glass wool filters.

c.1. The problem of sample volatility is real in the performance of fate studies and in some cases serves as the primary mechanism for the transport of organics from an aqueous environment.[4] Although the boiling points of DIMP and DCPD are high (174° C and 167° C, respectively), this physical property cannot be used to assume that the compounds will not volatilize from water. It has been found that compounds like DDT and other "non-volatiles" have appreciable volatilization rates.[5]

Our photochemical studies are performed in sealed systems so volatility is not a problem in performing this task. However, in microbial degradation studies where aeration is needed, test chemical losses due to volatilization could be great. We expect this effect would be greatest in the case of DCPD where low solubility indicates little association with solvent molecules.

To circumvent this problem, aeration is kept to a minimum and the water is monitored for its oxygen content to insure an adequate supply for biological oxidation. Also, gases exiting the flasks are passed through solid adsorbents (Tenax) to trap both the parent compound and volatile metabolites. The adsorbents are thermally desorbed directly into a gas chromatograph for analysis. This practice is done in both soil and aqueous solution microbial studies.

c.2. The adulteration of samples is avoided by adhering to "good laboratory practice." The collection, storage, and performance of all experiments are carried out in all glass systems and analytical samples are transferred in teflon-lined screw cap glass vials. All plastic containers and lids will be avoided due to phthalate and other plasticizer contamination as well as rubber and silicone linings in the apparatus.

c.3. See b.5. and b.3.

c.4. The analytical procedures for handling hydrophilic products were detailed in our proposal on page 5, paragraph 1. In reviewing the clarifications letter attachments for the analysis of the trimethylsilyl ester of methylphosphonic acid and the failure of Edgewood Arsenal to analyze this material by gas chromatography leads us to believe that there is a fault in their methodology and that trimethylsilylation is still a viable technique. This is based on the fact that isopropyl methylphosphonate is amenable to this technique and we have found in our laboratory that phosphonic acid readily forms a tri-TMS derivative that is analyzable by gas chromatography. We would expect that methylphosphonic acid would also be amenable to this technique.

Should this methodology fail, attempts will be made to form the dimethyl ester of methylphosphonic acid by treating the lyophilized residue with diazomethane in 9:1 diethyl ether:methanol. This procedure has worked well for over 20 carboxylic acids and phenols in our laboratory.

We also plan to examine aqueous solutions directly by high-pressure liquid chromatography using reverse-phase methods and uv detection at 210 nm to monitor the P=O absorption band. This region is also overshadowed by carboxyl absorption bands and it is difficult to predict the utility and sensitivity of this region at this time. This methodology will also be investigated on the lyophilized residues should the derivatization procedures fail.

c.5. SRI has the facilities and experienced personnel to perform the synthesis of radiolabeled compounds and would be pleased to submit a quotation for the synthesis of the test compounds at the Army's request. Our requirements for radiolabeled materials are estimated to be 5 mg of each material with a specific activity of 5-10 mc/m mole. We request that the radiochemical and chemical purity be 98%+. For non-labeled material, we require 50 grams of each chemical.

c.6. The objective of the photochemical studies is to determine photochemical half-lives for DCPD and DIMP. A primary consideration in treatment of any experimental data is the correct design and proper execution of the experiment. Some of the important factors in this regard are discussed in our original proposal, in items a.1, a.3, a.4, and a.5 above, and in reference 14. Experimental data will be plotted assuming the photochemical reaction kinetics are first-order in the test chemical. A computer least squares analysis will also be carried out using the first order assumption to obtain rate constants, standard deviations, and other statistical data. At the very low concentrations of the chemical in our experiments, which are expected in the aquatic environment, the absorbance of the chemical above 290 nm will be less than 0.02, i.e., less than 5% of the incident light is absorbed, and the reaction kinetics should therefore be of first order dependence[3,14,15,16].* If our kinetic data show significant deviation from

*See reference list in original proposal, page 29; and as added below.

15. R. G. Zepp. Quantum yields for reaction of pollutants in dilute aqueous solution. Environ. Sci. Technol. 12, 327-329 (1978).

16. Same as reference 14, except Part II, in press.

first order behavior, other kinetic analyses and experiments may be necessary, and further studies will be recommended to the Project Officer for discussion and approval.

If first order kinetic behavior is demonstrated, we will apply the rate constants obtained to estimate half-lives for photolysis in aquatic environments. If the absorption coefficients of DIMP and/or DCPD can be measured in the uv region above 290 nm, and if a quantum yield for photolysis at some wavelength is measured after discussions and written approval of the Project Officer, we will compute photolysis half-lives according to the method of Zepp and Cline.[3] This method has been briefly discussed on page 8 of our proposal (Quantum Yield and Calculation of Half-Life in Sunlight), but is repeated and expanded here to fully describe our data treatment.

If the photochemical reaction is first order in chemical, the rate of photolysis of a chemical at a wavelength is given by:

$$\frac{d[S]}{dt} = k_a \phi [S] = k_p [S]$$

where $[S]$ is the concentration of chemical

k_a is the rate constant for light absorption by the chemical

ϕ is the photochemical reaction quantum yield

k_p is the observed first order photolysis rate constant.

The rate constant k_a is a function of the absorption coefficient at the wavelength and a term proportional to the intensity of the incident light at this wavelength. The method of Zepp and Cline computes the sunlight photolysis rate constant by calculating and summing k_a over all wavelengths in the solar spectrum at which the chemical absorbs light. The computer program developed by Zepp and Cline is now operational at SRI, and allows calculation of direct photolysis rate constant and half-life in sunlight as a function of the time of day or time of year. Studies at EPA-ERL (Athens) and at SRI have found excellent agreement between half-lives measured for chemicals in sunlight when compared to the calculated half-lives.

If the data for calculation of half-lives by the method of Zepp and Cline cannot be obtained in laboratory measurements, we will estimate a photolysis half-life from the Hg lamp experiments (see pages 6 and 7) by analogy to other chemicals which we have previously studied. p-Cresol is one possible model compound since it has an uv absorption which weakly tails into the solar spectrum, and is similar to the spectra expected for DCPD and DIMP. Such half-life estimates will be very crude, but should suffice to document any low photochemical reactivity of these compounds. Although photolysis rates measured in sunlight are subject to wide variations due to cloud cover, time of year, air pollution and other factors, some experiments may be conducted to verify the calculated or estimated half-lives.

For those experiments which show no transformation of DCPD or DIMP, we will provide limiting rate constants, i.e., relating to shortest half-lives, that could have been measured in the experiment. Any extrapolation of data to environmental half-lives will be documented and any limitations, assumptions, or estimates in any calculations will be explicitly stated.

In the microbial degradation studies, the rate of transformation is a function of the degrading microorganism biomass concentration and the substrate concentration. When acclimated cultures are present at high population and a low level of substrate is introduced into the system, the microbial population does not change significantly when the substrate is used for their growth. Under these conditions, the degradation rate will be

$$-\frac{ds}{dt} = k_{b2}\, x_o\, S = k_b^1\, S$$

where S is the substrate concentration, x_o is the biomass concentration, k_{b2} is the degradation rate constant, and k_b^1 is a pseudo-first order rate constant. The k_b^1 rate constant and standard deviation will be determined at different biomass levels in well-stirred aquatic systems.

This constant (k_b^1) can be used to calculate the half-life of a pollutant at a given level of biomass and is an estimate of the persistance of a biodegradable pollutant in an acclimated environment. This will be a satisfactory rate expression for most biodegradations in natural contaminated waters containing a low pollutant level.

If in the environment where the biodegrading microbe concentration is low, the biomass level will change as the substrate is consumed. When the substrate is the growth limiting factor, the Monod model expression[6] can be applied as follows:

$$-\frac{ds}{dt} = \frac{\mu}{y}\, x = \frac{\mu m}{y}\left(\frac{sx}{k_s+s}\right) = k_b\, \frac{sx}{k_s+s}$$

where μ is the specific growth rate, μm is the maximum specific growth rate, y is the biomass yield constant, k_s is a constant equivalent to the concentration of chemical supporting a half-maximum growth rate (0.5 μm), and kb is a biodegradation rate constant.

Although Monod kinetics are based on the use of a pure culture and the rate of disappearance of a growth-rate limiting single substrate, these kinetic expressions can be used to obtain useful rate constants with mixed culture systems. It is possible to choose culture conditions that allow simplification of the Monod expression by making one of its variables constant, or one of its constants insignificant. The results of the simplification allow a straightforward estimation of the remaining constants. This can be achieved by batch fermentation procedures with low level of innocula or by continuous chemostat fermentation.

Where the constants are obtained from experimental data, they are incorporated into a computer program to estimate biodegradation half-lives (not first order) as a function of the substrate concentration.

We will choose the procedure and experimental conditions after biodegradation is found to be an important fate for the test chemicals in initial screening experiments. We will consult with the Project Officer for concurrence in the chosen procedures.

In the soil mineralization experiments, the application of Monod kinetics is difficult due to the complex makeup and heterogeneity of the soil. Therefore, the rate of measured $^{14}CO_2$ production will be used simply to obtain the time required to observe a 50% mineralization with a standard deviation at the experimental temperature.